(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 739 241 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
12.08.1998 Bulletin 1998/33

(21) Application number: 95906818.0

(22) Date of filing: 11.01.1995

(51) Int. Cl.[6]: B01L 7/00, G05D 23/13

(86) International application number:
PCT/US95/00413

(87) International publication number:
WO 95/18676 (13.07.1995 Gazette 1995/30)

(54) APPARATUS AND METHOD FOR THERMAL CYCLING NUCLEIC ACID ASSAYS

VORRICHTUNG UND VERFAHREN ZUR DURCHFÜHRUNG VON NUKLEINSÄUERETESTS MITTELS TEMPERATURZYKLEN

APPAREIL ET PROCEDE DE CYCLAGE THERMIQUE DE DOSAGES D'ACIDES NUCLEIQUES

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI NL

(30) Priority: 11.01.1994 US 179845

(43) Date of publication of application:
30.10.1996 Bulletin 1996/44

(73) Proprietor: Abbott Laboratories
Abbott Park, Illinois 60064-3500 (US)

(72) Inventors:
- ZUREK, Thomas, F.
River Forest, IL 60305 (US)
- HANLEY, Kathleen, A.
Grayslake, ILL. 60030 (US)
- PEPE, Curtis, J.
McHenry, IL 60050 (US)

(74) Representative:
Modiano, Guido, Dr.-Ing. et al
Modiano, Josif, Pisanty & Staub,
Baaderstrasse 3
80469 München (DE)

(56) References cited:
EP-A- 0 175 882
DE-A- 3 808 942
FR-A- 1 585 392
US-A- 3 053 454
WO-A-90/05947
DE-A- 4 028 891
US-A- 3 030 024

EP 0 739 241 B1

Typically, faster ramp time requires an inefficient high energy heater, sinc response time is valued mor than energy issues. In such cases, high power systems are utilized to alter temperature in a quick response cycle. On the oth r hand, highly efficient systems generally have slow intrinsic ramp speeds since the temperature changes are controlled using a low energy level.

Summary of the Invention

The subject invention is directed to a novel apparatus and a method for thermal cycling nucleic acid assays as claimed respectively in claims 1 and 5.

In the preferred embodiment, a plurality of constant volumetric flow fluid streams are provided in parallel. Each stream is of a different preselected temperature. Typically, a superheated stream (above maximum sample temperature) and a chilled or ambient stream are provided. Each stream is independently introduced into a diverter/mixer, where varying ratios are combined to produce an output stream of a selected intermediate temperature at a volumetric flow rate equal to the sum of the two individual input streams.

The term "constant volumetric flow" refers to the movement of a substantially fixed volume of fluid per unit time. The mass of this volume is dependent on the density of the fluid, but is unimportant to the invention as constant volume is desired. The volumetric flow rate is substantially constant moving in and out of the sample chamber regardless of the source. As is understood by those skilled in the art, absolute constancy is not achievable due to system inefficiencies and to variations in viscosity, humidity, temperature and other like factors. However, deviations resulting from these factors are of no consequence to the operation of the invention.

The present invention permits the use of constant temperature fluid streams to instantaneously control the temperature of a blended output stream. This allows the use of low energy heat exchangers which may have slow intrinsic ramp speeds since only a constant temperature output is required in order to produce each component of the blended stream. Rapid ramping of samples can nevertheless be obtained by instantaneously gating and blending the component streams, without having to alter the temperature of the entire body of bathing fluid. Therefore, the method and apparatus of the subject invention can greatly reduce equipment costs and energy consumption, while at the same time increasing the overall responsiveness of the system.

In the preferred embodiment of the invention, the fluid medium of choice is air. Typically, a first stream of air is heated to a selected temperature, substantially above the highest target temperature desired for the reaction sample. At least one additional stream of air is provided, which is typically substantially lower than the coolest target temperatur required for th sample. Contr lled, variable ratios of the heated airstream and the cooled air stream are then introduced into a mixing chamber via gating means for producing a blended air stream. By controlling the gate and the ratio of the first air stream to the s cond air stream, almost instantaneous changes in temperature of the blended air stream can be achieved, providing similarly r sponsive temperature changes in the sample chamber.

In one aspect of the invention, the blended temperature streams may be set at the desired target level and introduced directly into the controlled environment of the sample for altering the control temperature. In another aspect of the invention, the ramp speed for altering the temperature of the sample can be further accelerated by utilizing a blended air stream initially at a temperature substantially beyond the target temperature for the sample. This permits the sample to rapidly heat to a point near its target temperature, at which point the blended air stream is altered to maintain a target temperature. The sequencing of this alternative is dependent upon the hysteresis of the bath vessel and the sample. As the sample approaches the target temperature, the blended air streams are adjusted to define a chamber temperature adapted for maintaining the sample at the target temperature for the specified period of time. After the sample has been held at the target temperature for the specified period of time, the blended air stream is cooled by changing the ratio of heated air to cooled air, again almost instantaneously changing the chamber temperature, permitting the sample temperature to be rapidly altered.

By utilizing the thermal cycling method and apparatus of the subject invention, it has been found that the sample can be held at the target temperature for periods of less than one second in duration by diverting the constant temperature air streams and controlling the ratios thereof to control the blended air stream which is introduced into the sample chamber.

Utilizing air as the medium of choice eliminates disposal and recycling problems since room air can be used for both air streams. One particular advantage of the subject invention is the elimination of inefficient, high energy heating/cooling systems since the slow intrinsic ramp time of such a heating and cooling system is no longer an issue. The invention has been found to be particularly useful in samples where ramp time is a critical factor and temperature change has to be accomplished in very precise, controlled time intervals.

It is, therefore, a feature of the subject invention to provide a thermal cycling device and method which permits rapid, controlled ramping of assay samples and rapid temperature change of sample environments.

Other objects and features of the invention will be readily apparent from the accompanying drawings and detailed description of the preferred embodiments.

Brief Description of the Drawings

Fig. 1 is a flow diagram of a fluid control circuit in accordance with the subject invention.

stant temperature, the intrinsic ramp time of the chilling or cooling apparatus is not critical. The constant temperature cooled air is then delivered at transfer line 26. Optionally, the chiller is omitted and air at ambient temperature is used in its place.

In the embodiment of Fig. 2, a pair of damper systems 28 and 30 are placed in parallel and each has an input port 32 adapted for receiving the stream in lines 18 and 26, respectively. Each damper also includes an output port 34 and an exhaust or return port 36.

Each damper system has a diverter gate system as diagrammatically illustrated at 38. In operation, treated air from the heater or the chiller enters the respective damper via the input port 32. When the gate 38 is shifted to block exhaust port 36, all air entering the diverter through port 32 is exited through port 34 and into the common conduit 40. It will be recognized that the ratio of chilled air to heated air can be controlled by selectively controlling the position of the respective gates 38. The ratio can vary from zero percent (0%) chilled air and one hundred percent (100%) heated air to zero percent (0%) heated air and one hundred percent (100%) chilled air, since the position of the gates are infinitely variable. This permits the commingled air in the combined fluid stream of conduit 40 to be of any select temperature between the lowest temperature provided by the chilled air and the highest temperature provided by the heated air.

The blowers 12 and 20 are in parallel as are the heater 16 and the chiller 24. The gates 38 are controlled to provide a combined fluid stream exiting through the respective ports 34. This combined or commingled stream is present in the common conduit 40 and is at a constant volumetric flow rate. Thus, the blended stream in line 40 is controlled to generate the entire temperature range required for thermal cycling the samples.

The commingled air stream is then introduced into a mixer 42 where it is blended to produced a homogeneous, controlled temperature fluid flow in line 44. The flow may be controlled through a series of baffles in the manner well known to those who are skilled in the art to assure a smooth flowing, homogeneous flow. This blended stream is introduced via the transfer conduit 44 into the cycling or sampling chamber 10.

In the preferred embodiment, the sample chamber 10 may include means for supporting one or more sample specimens. By way of example, it has been found that suspension of the specimens on a suitable suspension wire or the like minimizes contact of the specimen vials with any potential surface that could act as either an insulator or a heat-sink and affect the ramp up and ramp down times of the specimen during the thermal cycling. In the preferred embodiment, the samples may be suspended in the sample chamber from a suitable hanger such as a wire rack or the like. Suspended sample tubes may also serve adequately as baffles, thereby obviating the mixer 42.

Th fluid stream through the sample chamber is exited via the transfer conduit 46. The specimen is continuously in contact with a moving fluid stream having a constant volumetric flow rate with an almost instantaneously variable temperature. The temperature may be rapidly changed by shifting the controlled gates 38 in the damper systems 28 and 30, respectively, the temperature of the fluid stream 44 being nearly instantaneously responsive to the repositioning of the gates. In the embodiment of Fig. 2, the fluid exited from chamber 10 via conduit 46 is introduced to the input sides of the blowers 12 and 20. Likewise, the excess exhaust fluid exhausted via the respective ports 36 of the damper systems 28 and 30 is reintroduced into the fluid stream via recirculating paths 48 and 50, respectively. The recirculation paths are coupled to the exhaust paths 46, as diagrammatically shown at 52 and 54, respectively. The relative intake of blowers 12, 20 from conduit 46 is proportional to the output from each blower which is gated through dampers 28, 30 to the mixer 42 and sample chamber 10, thus maintaining a substantially constant volumetric flow rate throughout the closed system.

An alternative embodiment is shown in Fig. 3. This has been found to be the preferred embodiment of the invention, since the addition of blower 60 has been found to allow the system to operate with a minimum waste of energy in the heater (16) and chiller (24). The additional pump or blower 60 has been inserted in the system in parallel with the pump or blowers 12 and 20, with input from the exhaust system conduit 46 and output being introduced directly into the mixer 42 via the conduit 62, in parallel with the commingled stream from conduit 40.

By providing proper gating or dampening at mixer 42, the blower 60 works in conjunction with the blowers 12 and 20 to permit heated or chilled air to be introduced into the mixer 42 only as required while maintaining a constant volumetric slow rate through the mixer 42 and sample chamber 46. For example, when a sample target temperature has been reached and must be maintained, dampers 28, 30 can both be closed down to exhaust the majority of the output of blowers 12 and 20 through their recycle paths 48 and 50. This causes blower 60 to intake the majority of output from sample chamber 10 via conduit 46, and to re-circulate it back to sample chamber 10 without a temperature adjustment. This process continues until the temperature monitor calls for a decrease or increase in temperature, where upon the chiller damper 30 or heater damper 28 is opened to make the adjustment. It has been found that the addition of blower 60 has a minimum impact on the temperature cycling and response time of the system and allows heated or chilled air to be introduced into the sample chamber 46 only as required.

A third embodiment utilizing a single sample damper 70 is shown in Fig. 4. This embodiment is particularly useful when system cost considerations are a factor. As there shown, the damper 70 includes an ambient air input port 72 and a heated air input port 74.

As in the prior embodiments, the blower 12 is associated with the heater 16 via the input conduit 14 for producing heated air in conduit 18 which then is introduced via the input port 74 into the damper 70. In this embodiment, a second blower 80 is adapted for receiving ambient exterior air, as indicated by arrow A for introducing a constant flow of ambient air via conduit 82 into the ambient air input port 72. The damper 70 includes a gate 38 operating in the same manner previously described for controlling the ratio of heated air and ambient air which is output via the output port 84 into the common or blended air conduit 40. The blended air is introduced into the mixer 42, as in the previous embodiments, for producing a blended, having a constant volumetric fluid stream flow rate on conduit 44 for introduction into the sample chamber 10. The exhaust system 46 is connected directly to the heated input blower 12 via conduit 86, with an open exhaust at 88 to the room. Because of the open exhaust 88, this embodiment also operates with a minimum of back pressure, assuring that a constant volumetric flow rate fluid stream is produced. This system is ideal when the lowest temperature the samples must achieve in the cycling chamber 10 is at or above ambient temperature. Of course, it will be understood that a chilled air could be introduced into port 72 of the single damper 70 to lower the low temperature end of the ramp.

Dampers can be any known device. The diverters of the dampers may be controlled by standard equipment, such as stepper motors under computer control.

The drawing figures are diagrammatic only, especially with regard to conduits. In actual construction, such conduits are minimized so as to decrease dead space volume and to assure the essentially instantaneous effect of a gating change in fluid flow.

As mentioned, the pumps or blowers used in this invention should be of a type that moves a constant volume of fluid against a constant backpressure. Such pumps are exemplified by the class of pumps known as "centrifugal" pumps or blowers. They are characterized by an ability to produce a constant volumetric flow, and by an ability to produce an output that varies inversely with the pressure against which they pump. Thus, in Figure 3 for example, when one desires to raise the temperature of the fluid stream bathing the samples, the gate from the heater 16 is opened to flood the common conduit 40 with heater fluid. This causes blower 60 to encounter increased backpressure and to reduce its output automatically.

Experiments establishing the viability of the system have been performed with good results, wherein the air stream was able to be maintained at a constant flow of 1.3 m^3/min (45.63 CFM), when utilizing a heat gun, model HG501A, available from Master Appliance Company, Racine, Wisconsin, with a maximum temperature of approximat ly 126°C at 1,680 watts. The nozzle air velocity was maintained at 914 m/min (3,000 fpm), with a nozzle exit diameter of 1.67 in. to achieve a flow of 0.7 m^3/min (24.7 SCFM (standard cubic feet per minute)).

The experimental samples contained water in an Eppendorf® brand .5 ml. spherical bottom polypropylene microcentrifug tube with an attached lid, as is standard in the industry. Each tube contained 60 microliters of water.

The lid was drilled to accept a pair of thermocouple wires. The thermocouple used was Omega PN TT-T-30, T Type, 0.025 cm (.010 inch) wire diameter. The junction at the cap was welded with a weld bead diameter of approximately 0.076 cm (0.030) inches. The junction was positioned at approximately the center of the liquid in the tube. An Omega Digital Thermometer, Model HH-73T, was used to monitor the sample temperature. During the experiment, the sample temperature was at an initial temperature of 22°C and could be brought to a peak temperature of 100°C in a period of approximately thirty (30) seconds. The vial could then be cooled from the peak temperature of 100°C to the initial temperature of 22°C in a period of approximately 4.5 minutes using ambient air as the cooling medium.

It was further established by increasing the heated temperature level to a level five times higher than the target temperature and bringing the chamber temperature to a level initially higher than the target temperature for the vial, that the ramp speed could be substantially increased. By injecting 126°C air into the chamber, the sample could be heated from 50° C to 85° C within 12 to 15 seconds. Likewise, by injecting cooling air at a substantially lower temperature than the target temperature, for example, 22° air, the sample was cooled from 85°C to 50°C in approximately 60 to 75 seconds, greatly increasing the cooling ramp speed.

In the exemplary embodiments, it is contemplated that a fluid surface area of the sample chamber will be constructed of a material having minimum "heat sink" characteristics, further assuring that the cycling chamber 10 is quickly responsive to a change in gate position by gates 38 of the dampers 28 and 30. Of course, insulation and selection of materials have an important role in these issues.

Phase diagrams demonstrating operating cycles for the thermal cycling system as described herein are illustrated in Figs. 5 and 6. With specific reference to Fig. 5, the temperature axis is vertical and the time duration is shown on the horizontal line. TR represents room temperature and is presumed to be the initial temperature of the sample. In the example illustrated, the chilled air is at a constant temperature substantially below room temperature, as indicated by TC. The heated air stream is at a constant temperature substantially above room temperature, as indicated by TH.

It will be understood that in certain applications, ambient room temperature air may be utilized in lieu of the chilled air or the heated air or in combination with both streams. Using Fig. 2 as an example, the sample is placed in the chamber 10 at time t0, at room temperature TR. The thermal cycling is then initiated at time t0

by adjusting the baffle gates 38 in dampers 28 and 30 to produce a blended air stream of a temperature corresponding to the target sample temperature TS. The sample temperature then begins to rise, as indicated and reaches the target temperature TS at time t2. The sample will be maintained at this temperature for as long as the blended air stream is held at the target level, or until t3. When the sample has been heated for the appropriate period of time as indicated at t3, the baffle gates in dampers 28 and 30 are adjusted to bring the blended air stream temperature down to either room temperature TR or another selected temperature and the sample begins to cool down, reaching the lower blended air stream temperature at time t4, where it will stay until the temperature of the blended air stream is again adjusted or the sample is removed from the sample chamber 10.

A super heating and/or super cooling cycle is illustrated in Fig. 6. This cycle method is useful when fast ramp up and ramp down times are desirable. Again using the embodiment of Fig. 2 as an example, a sample is placed in chamber 10 at room temperature TR at time t0. The cycle begins at time t5, when the baffle gates in dampers 28 and 30 are adjusted to produce a blended air stream at a temperature TA, which is substantially higher than the target sample temperature TS. Of course, TA may be as high as TH. The sample temperature begins to rapidly rise in response to its contact with the super heated air. As the sample temperature approaches the target temperature TS, the baffles are adjusted to reduce the blended air temperature to TS as indicated at time t6, with the sample reaching target temperature TS at time t7. The sample will stay at this temperature for as long as the blended air is maintained at the target temperature TS, or until t8, as indicated. Where it is desired to rapidly cool the sample, the blended air can be adjusted to a super cooled to a temperature TB, substantially below room temperature TR as indicated at time t8. TB may be as low as TC. As the sample approaches room temperature TR or another preselected final temperature, the blended air temperature is adjusted as indicated at time t9.

The method as demonstrated in Fig, 6 relies on the natural hysteresis of the sample to permit either or both super heating and super cooling to maximize ramp times. Specifically, by overshooting the target sample temperature TS, the transition time between TR and TS is greatly accelerated. This may be utilized to quickly elevate the sample temperature as between time points t5 and t6 and/or to quickly reduce sample temperature as between t8 and t9. This permits the cycling of samples requiring quick temperature change, utilizing constant temperature streams as indicated by TH and TC. The gap between times t6 and t7 and/or between t8 and t9 is dictated by the natural hysteresis of the sample. Typically, a greater differenc between the target sample temperature TS and the overshoot temperature TA or TC results in a faster ramp speed. This method not only permits control of the sample temperature throughout the cycle but also permits control f the transition time between a plurality of target points.

While certain features and embodim nts of th invention have been described in detail herein, it will be readily apparent that the invention encompasses all modifications and enhancements within the scope of the following claims, as provided for by Art. 69 of the EPC.

Where technical features mentioned in any claim are followed by reference signs, those reference signs have been included for the sole purpose of increasing the intelligibility of the claims and accordingly, such reference signs do not have any limiting effect on the scope of each element identified by way of example by such reference signs.

Claims

1. An apparatus for thermal cycling nucleic acid assays, comprising:

    a. a sample chamber (10) for holding a nucleic acid specimen;
    b. a first fluid source (12) for providing a first fluid stream maintained at a first predetermined temperature;
    c. a second fluid source (20) for providing a second fluid stream which is separate from said first fluid stream and which is maintained at a second predetermined temperature;
    d. a mixing device (42) for receiving independently both said first and said second fluid streams from said first and second fluid sources (12, 20), and for commingling said first and second fluid streams in variable, controlled ratios for producing a homogeneous fluid stream of a preselected temperature in a range between said first and second predetermined temperatures; said mixing device (42) being in fluid communication with said sample chamber (10) so that said homogenous fluid stream having the temperature obtained from the mixing of said two separate first and second fluid streams is fed into the said sample chamber;
    e. means (28, 30; 70) for varying the ratio of first and second fluid streams; and
    f. an exit system (46) for removing the blended fluid stream from said sample chamber (10).

2. The apparatus according to claim 1, further including:

    a heater (16) for heating said first fluid stream to a temperature above ambient temperature before said fluid stream enters the mixing device (42);
    a chiller (24) for cooling said second fluid

stream to a temperature below ambient temperature before said second fluid stream enters the mixing device (42);

means (48, 50) for maintaining both said first and said second fluid streams at a constant volumetric flow rate; and

a control system associated with the mixing device (42) for selectively varying the ratio of said first fluid stream to said second fluid stream in the blended fluid stream.

3. The apparatus according to claims 1 or 2, wherein said mixing device further comprises a mixing chamber (42) having input ports for receiving said first and second fluid streams, and a single exit port in communication with a transfer system (44) for introducing a blended fluid stream to said sample chamber (10); and wherein said means (28, 30) for varying the ratio comprises a gate system (38) in communication with said mixing chamber (42) for selectively restricting the respective flow of one or both of said first and second fluid streams.

4. The apparatus according to claim 3, wherein there are two input ports in parallel and wherein said gate system (38) comprises a single diverter valve in simultaneous communication with each of said ports, or

wherein said gate system (38) comprises a pair of diverter valves, one each in communication with one of said input ports.

5. A method for thermal cycling a sample comprising the steps of:

a. providing a plurality of separate component fluid streams each from a distinct source maintained at a constant temperature and blending them so that a homogenous composite fluid stream of a preselected temperature is obtained

b. directing and introducing the flow of said composite stream having said obtained preselected temperature directly into the environment of said sample to achieve a first sample-target temperature;

c. maintaining said first sample-target-temperature for a preselected period;

d. adjusting said composite stream to achieve a second sample-target-temperature, wherein said adjusting is achieved by selectively blending in varying ratios said component fluid streams; and

e. maintaining said second sample-target-temperature for a preselected period.

6. The method of claim 5, wherein said composite stream of step (a) has a temperature substantially the same as said first sample-target-temperature.

7. The method of claim 5, wherein said composite stream of step (a) initially has a temperature disparate from said first sample-target-temperature and after a time sufficient to substantially achieve said first sample-target-temperature, said composite stream is altered to a temperature substantially the same as said first sample target-temperature.

8. The method of one or more of claims 5-7, wherein said adjusted composite stream of step (d) has a temperature substantially the same as said second sample-target-temperature.

9. The method of one or more of claims 5-7, wherein said adjusted composite stream of step (d) initially has a temperature disparate from said second sample-target-temperature and after a time sufficient to substantially achieve said second sample-target temperature, said adjusted composite stream is altered to a temperature substantially the same as said second sample-target temperature.

10. The method of one or more of claims 5-9, wherein said plurality of component fluid streams comprises at least one component fluid stream at a temperature higher than ambient temperature, and at least one component fluid stream at a temperature lower than ambient temperature.

11. The method of one or more of claims 5-9, wherein said plurality of component fluid streams comprises three component streams wherein one component fluid stream is at a temperature higher than ambient temperature, one component fluid stream is at a temperature lower than ambient temperature and one component fluid stream is fluid recirculated into said sample environment.

12. The method of one or more of claims 5-11, wherein said first sample-target temperature and said second sample-target-temperature are repeatedly achieved.

13. The method of one or more of claims 5-12, wherein said sample is a nucleic acid and wherein said first and second sample target temperatures are first and second nucleic acid target-temperatures, respectively.

Patentansprüche

1. Vorrichtung zur Durchführung von Nukleinsäureassays mithilfe von Temperaturzyklen, die folgendes umfaßt:

a. eine Probenkammer (10), um eine Nuklein-

säureprobe aufzunehmen;

b. eine erste Fluidquelle (12), um einen ersten Fluidstrom bereitzustellen, der auf einer vorbestimmten ersten Temperatur gehalten wird;

c. eine zweite Fluidquelle (20), um einen zweiten Fluidstrom bereitzustellen, der vom ersten Fluidstrom getrennt ist und der auf einer zweiten vorbestimmten Temperatur gehalten wird;

d. eine Mischvorrichtung (42), um unabhängig sowohl den ersten als auch zweiten Fluidstrom aus der ersten und zweiten Fluidquelle (12, 20) aufzunehmen und um den ersten und zweiten Fluidstrom in veränderbaren, gesteuerten Verhältnissen zu vermischen, damit ein homogener Fluidstrom einer vorab ausgewählten Temperatur in einem Bereich zwischen der ersten und der zweiten vorbestimmten Temperatur erzeugt wird; wobei die Mischvorrichtung (42) in Fluidverbindung zu der Probenkammer (10) steht, so daß der homogene Fluidstrom, der die Temperatur aufweist, die aus dem Vermischen der zwei getrennten ersten und zweiten Fluidströme erhalten wird, der Probenkammer zugeführt wird;

e. ein Mittel (28, 30, 70), um das Verhältnis des ersten und zweiten Fluidstroms zu verändern; und

f. ein Austrittsystem (46), um den vermengten Fluidstrom aus der Probenkamner (10) zu entfernen.

2. Vorrichtung nach Anspruch 1, die des weiteren folgendes umfaßt:

einen Heizapparat (16), um den ersten Fluidstrom auf eine Temperatur zu erhitzen, die über der Umgebungstemperatur liegt, bevor der Fluidstrom in die Mischvorrichtung (42) eintritt;

einen Kühlapparat (24), um den zweiten Fluidstrom auf eine Temperatur zu kühlen, die unter der Umgebungstemperatur liegt, bevor der zweite Fluidstrom in die Mischvorrichtung (42) eintritt;

ein Mittel (48, 50), um sowohl den ersten als auch den zweiten Fluidstrom auf einer konstanten volumetrischen Fließgeschwindigkeit zu halten; und

ein mit der Mischvorrichtung (42) verknüpftes Steuersystem, um das Verhältnis des ersten Fluidstroms und des zweiten Fluidstroms im vermengten Fluidstrom selektiv zu verändern.

3. Vorrichtung nach den Ansprüchen 1 oder 2, worin die Mischvorrichtung des weiteren eine Mischkammer (42), die zum Aufnehmen des ersten und zweiten Fluidstroms über eine Einlaßöffnung verfügt, und eine inzelne Austrittsöffnung umfaßt, die in Verbindung mit einem Übertragungsystem (44) steht, damit ein vermengter Fluidstrom in die Probenkammer (10) eingeschleust wird; und worin das Mittel (28, 30) zum Verändern des Verhältnisses ein Schleusensystem (38) umfaßt, das mit der Mischkammer (42) in Verbindung steht, um den jeweiligen Fluß des ersten oder des zweiten Fluidstroms oder beider selektiv einzuschränken.

4. Vorrichtung nach Anspruch 3, worin sich zwei parallele Einlaßöffnungen befinden und worin das Schleusensystem (38) ein einzelnes Abzweigventil in gleichzeitiger Verbindung mit jeder der Öffnungen umfaßt, bzw.

worin das Schleusensystem (38) ein Paar von Abzweigventilen umfaßt, von denen ein jedes mit einer Einlaßöffnung in Verbindung steht.

5. Verfahren für die Wärmezyklenbehandlung einer Probe, das folgende Schritte umfaßt:

a. das Bereitstellen und Vermengen einer Mehrzahl von getrennten Fluidstrom-Bestandteilen, jeder aus einer anderen Quelle, die auf einer konstanten Temperatur gehalten wird, so daß ein homogener zusammengesetzter Fluidstrom einer vorab ausgewählten Temperatur erhalten wird;

b. das Lenken und Einbringen des Flusses des zusammengesetzten Stroms, der über die erhaltene vorab ausgewählte Temperatur verfügt, unmittelbar in die Umgebung der Probe, um eine erste Proben-Solltemperatur zu erzielen;

c. das Beibehalten der ersten Proben-Solltemperatur über eine vorab ausgewählte Zeitspanne;

d. das Einstellen des zusammengesetzten Stroms, um eine zweite Proben-Solltemperatur zu erzielen, worin das Einstellen durch das selektive Vermengen erreicht wird, indem die Fluidstrombestandteile in wechselnden Verhältnissen selektiv vermischt werden; und

e. das Beibehalten der zweiten Proben-Ziel-Temperatur über eine vorab ausgewählte Zeitspanne.

6. Das Verfahren nach Anspruch 5, worin der zusammengesetzte Strom aus Schritt (a) über eine Temperatur verfügt, die im wesentlichen dieselbe wie die erste Proben-Solltemperatur ist.

7. Das Verfahren nach Anspruch 5, worin der zusammengesetzte Strom aus Schritt (a) anfänglich eine Temperatur aufweist, die sich von der ersten Proben-Solltemperatur unterscheidet, wobei nach einer Zeitspanne, die ausreicht, um die erste Proben-Solltemperatur im wesentlichen zu erreichen, der zusamm ngesetzte Strom auf eine Temperatur

verändert wird, die im wesentlichen die gleiche wie die erste Proben-Solltemperatur ist.

8. Das Verfahren von einem oder mehreren der Ansprüche 5-7, worin der eingestellte zusammengesetzte Strom aus Schritt (d) eine Temperatur aufweist, die im wesentlichen die gleiche wie die zweite Proben-Solltemperatur ist.

9. Das Verfahren nach einem oder mehreren der Ansprüche 5-7, worin der eingestellte zusammengesetzte Strom aus Schritt (d) anfänglich eine Temperatur aufweist, die sich von der zweiten Proben-Solltemperatur unterscheidet, wobei nach einer Zeitspanne, die ausreicht, um im wesentlichen die zweite Proben-Solltemperatur zu erreichen, der eingestellte zusammengesetzte Strom auf eine Temperatur verändert wird, die im wesentlichen dieselbe wie die zweite Proben-Solltemperatur ist.

10. Das Verfahren nach einem oder mehreren der Ansprüche 5-9, worin die Mehrzahl der Fluidstrom-Bestandteile mindestens einen Fluidstrom-Bestandteil bei einer Temperatur umfaßt, die höher als die Umgebungstemperatur ist, und mindestens einen Fluidstrom-Bestandteil bei einer Temperatur umfaßt, die niedriger als die Umgebungstemperatur ist.

11. Das Verfahren nach einem oder mehreren der Ansprüche 5-9, worin die Mehrzahl von Fluidstrom-Bestandteilen drei Strom-Bestandteile umfaßt, worin ein Fluidstrom-Bestandteil bei einer Temperatur liegt, die höher als die Umgebungstemperatur ist, ein Fluidstrombestandteil bei einer Temperatur liegt, die niedriger als die Umgebungstemperatur ist, und ein Fluidstrom-Bestandteil Fluid ist, das in die Probenumgebung zurückgeführt wird.

12. Das Verfahren nach einem oder mehreren der Ansprüche 5-11, worin die erste Proben-Solltemperatur und die zweite Proben-Solltemperatur wiederholt erreicht werden.

13. Das Verfahren nach einem oder mehreren der Ansprüche 5-12, worin die Probe eine Nukleinsäure ist und worin die erste und die zweite Proben-Solltemperatur jeweils eine erste und zweite Nukleinsäure-Solltemperatur sind.

Revendications

1. Appareil pour cyclage thermique de dosages d'acide nucléique comprenant :

   a. une chambre d'échantillon (10) destiné à contenir un spécimen d'acid nucléique ;
   b. une première source de fluide (12) destiné à délivrer un premier courant de fluide maintenu à une première température prédéterminée ;
   c. une seconde source de fluide (20) destinée à délivrer un second courant de fluide qui est distinct dudit premier courant de fluide et qui est maintenu à une seconde température prédéterminée ;
   d. un dispositif (42) de mélange destiné à recevoir indépendamment à la fois ledit premier et ledit second courants de fluide desdites première et seconde sources de fluide (12, 20), et à mélanger ensemble lesdits premier et second courants de fluide dans des rapports variables, commandés, pour produire un courant de fluide homogène d'une température prédéterminée dans une plage entre lesdites première et seconde températures prédéterminées ; ledit dispositif de mélange (42) étant en communication fluidique avec ladite chambre d'échantillon (10), de façon à délivrer, dans ladite chambre d'échantillon, ledit courant de fluide homogène ayant la température obtenue par le mélange desdits deux premier et second courants de fluide distincts ;
   e. un moyen (28, 30 ; 70) destiné à faire varier le rapport des premier et second courants de fluide ; et
   f. un système de sortie (46) destiné à faire sortir, de ladite chambre d'échantillon (10), le courant de fluide mélangé.

2. Appareil selon la revendication 1, comprenant en outre :

   un réchauffeur (16) destiné à chauffer ledit premier courant de fluide à une température au-dessus de la température ambiante avant que ledit courant de fluide n'entre dans le dispositif (42) de mélange ;
   un refroidisseur (24) destiné à refroidir ledit second courant de fluide à une température au-dessous de la température ambiante avant que ledit second courant de fluide n'entre dans le dispositif (42) de mélange ;
   un moyen (48, 50) destiné à maintenir à la fois ledit premier et ledit second courants de fluide à un débit volumétrique constant ; et
   un système de commande associé au dispositif (42) de mélange pour faire varier sélectivement le rapport dudit premier courant de fluide audit second courant de fluide dans le courant de fluide mélangé.

3. Appareil selon la revendication 1 ou 2, dans lequel ledit dispositif de mélange comprend en outre une chambre (42) de mélange ayant des orifices d'entrée destinés à recevoir lesdits premier et

second courants de fluide, et un unique orifice de sortie en communication avec un systèm (44) de transfert destiné à introduir un courant de fluide mélangé dans ladite chambre (10) d'échantillon ; et dans lequel ledit moyen (28, 30) destiné à faire varier le rapport comprend un système (38) de porte en communication avec ladite chambre (42) de mélange pour limiter sélectivement le flux respectif de l'un ou des deux desdits premier et second courants de fluide.

4. Appareil selon la revendication 3, dans lequel il y a deux orifices d'entrée en parallèle et dans lequel ledit système (38) de porte comprend une unique soupape de partition en communication simultanée avec chacun desdits orifices ; ou

dans lequel ledit système (38) de porte comprend une paire de soupapes de partition, chacune en communication avec l'un desdits accès d'entrée.

5. Procédé de cyclage thermique d'un échantillon comprenant les étapes :

a. de délivrance de plusieurs courants de fluide composants distincts, chacun provenant d'une source distincte maintenue à une température constante, et de mélange de ceux-ci de façon à obtenir un courant de fluide composite homogène d'une température choisie préalablement ;

b. d'envoi et d'introduction du flux dudit courant composite, ayant obtenu ladite température choisie préalablement, directement dans l'environnement dudit échantillon pour atteindre une première température cible d'échantillon ;

c. de maintien de ladite première température cible d'échantillon pendant une période choisie préalablement ;

d. de réglage dudit courant composite pour atteindre une seconde température cible d'échantillon, ledit réglage s'obtenant en mélangeant sélectivement, dans des rapports variables, lesdits courants de fluide ; et

e. de maintien de ladite seconde température cible d'échantillon pendant une période choisie préalablement.

6. Procédé selon la revendication 5, dans lequel ledit courant composite de l'étape (a) a une température qui est sensiblement la même que ladite première température cible d'échantillon.

7. Procédé selon la revendication 5, dans lequel ledit courant composite de l'étape (a) a, initialement, une température distincte de ladite première température cible d'échantillon et, après un temps suffisant pour atteindre sensiblement ladite première température cible d'échantillon, ledit courant composite est modifié pour avoir sensiblement une température qui est la même que ladite première température cible d'échantillon.

8. Procédé selon une ou plusieurs des revendications 5 à 7, dans lequel ledit courant composite réglé de l'étape (d) a une température qui est sensiblement le même que ladite seconde température cible d'échantillon.

9. Procédé selon une ou plusieurs des revendications 5 à 7, dans lequel ledit courant composite réglé de l'étape (d) a, initialement, une température distincte de ladite seconde température cible d'échantillon et, après un temps suffisant pour atteindre sensiblement ladite seconde température cible d'échantillon, ledit courant composite réglé est modifié pour avoir sensiblement une température qui est la même que ladite seconde température cible d'échantillon.

10. Procédé selon une ou plusieurs des revendications 5 à 9, dans lequel lesdits plusieurs courants de fluide composants comprennent au moins un courant de fluide composant à une température plus élevée que la température ambiante, et au moins un courant de fluide composant à une température plus basse que la température ambiante.

11. Procédé selon une ou plusieurs des revendications 5 à 9, dans lequel lesdits plusieurs courants de fluide composants comprennent trois courants composants, un courant de fluide composant étant à une température plus élevée que la température ambiante, un courant de fluide composant étant à une température plus basse que la température ambiante et un courant de fluide composant étant un fluide ayant recirculé dans ledit environnement d'échantillon.

12. Procédé selon une ou plusieurs des revendications 5 à 11, dans lequel ladite première température cible d'échantillon et ladite seconde température cible d'échantillon sont obtenues de façon répétitive.

13. Procédé selon une ou plusieurs des revendications 5 à 12, dans lequel ledit échantillon est un acide nucléique et dans lequel lesdites première et seconde températures cibles sont, respectivement, des première et seconde températures cibles d'acide nucléique.

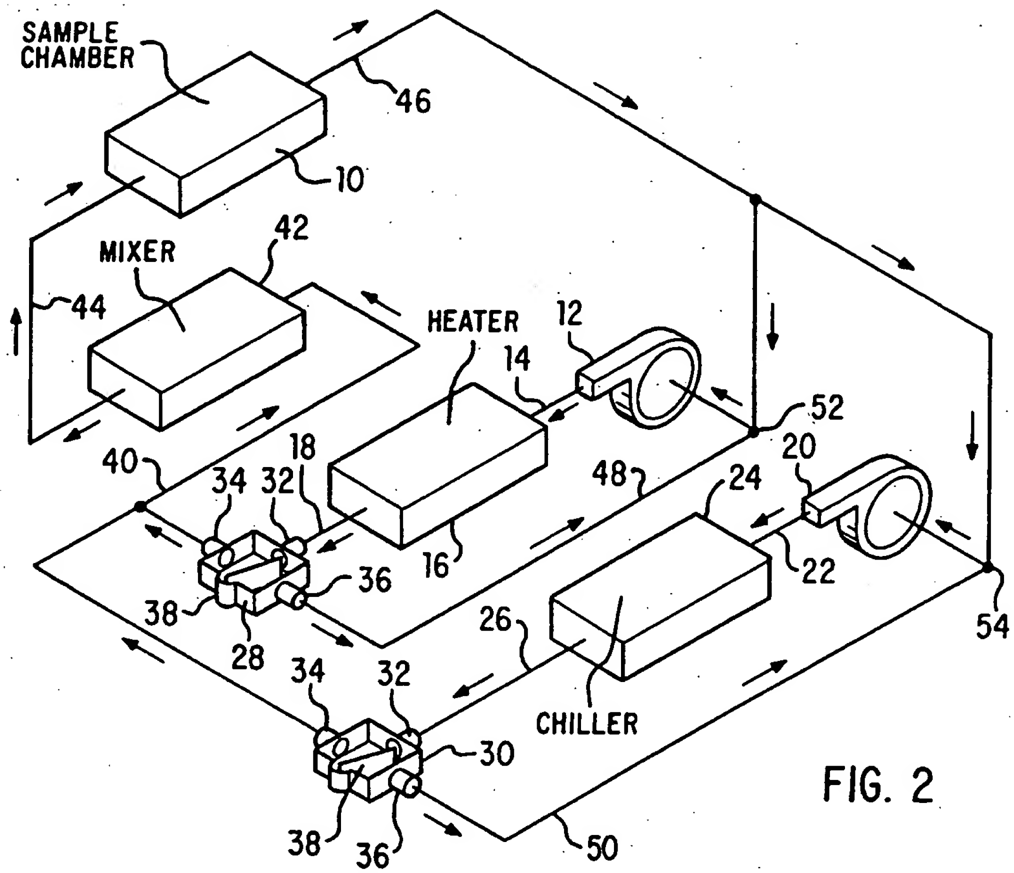

SAMPLE CHAMBER
10
46
MIXER
42
44
HEATER
12
14
52
20
40
18
34
32
48
24
16
22
36
38
28
26
54
34
32
CHILLER
30
38
36
50

FIG. 2

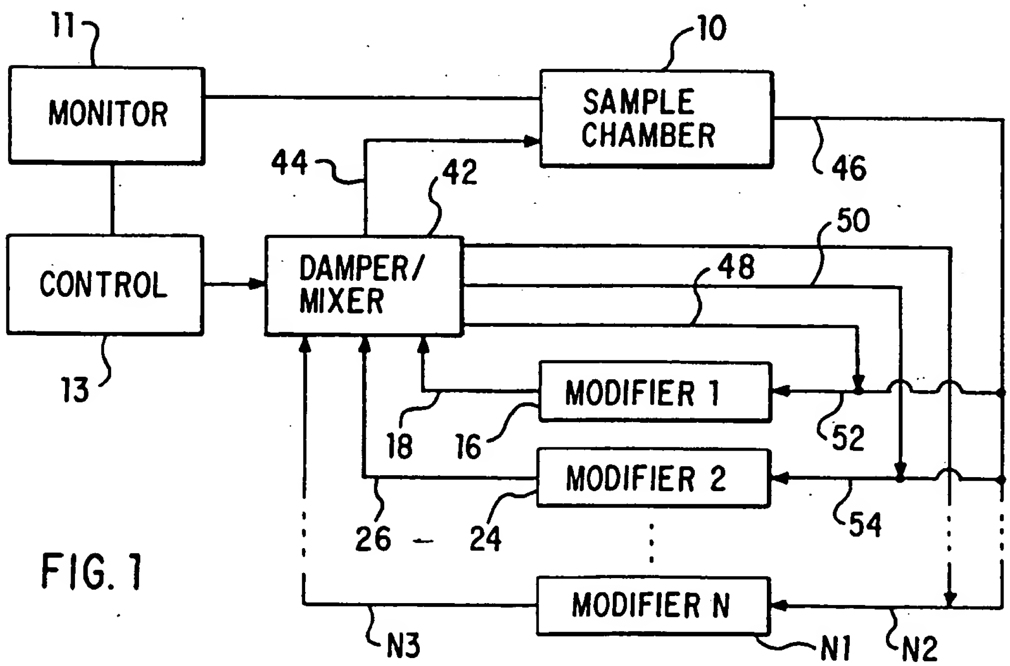

11
MONITOR
10
SAMPLE CHAMBER
46
44
42
50
CONTROL
DAMPER/ MIXER
48
13
MODIFIER 1
18
16
52
MODIFIER 2
26
24
54
MODIFIER N
N3
N1
N2

FIG. 1

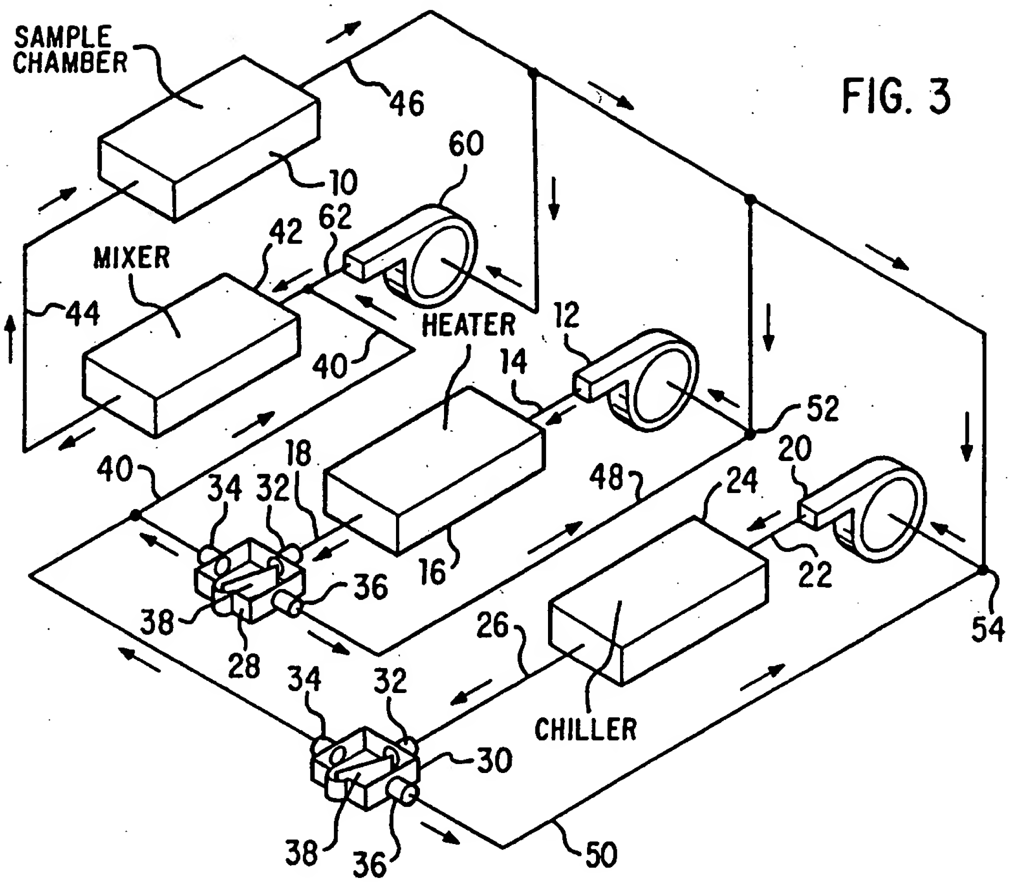

SAMPLE CHAMBER
MIXER
HEATER
CHILLER
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
60
62

FIG. 3

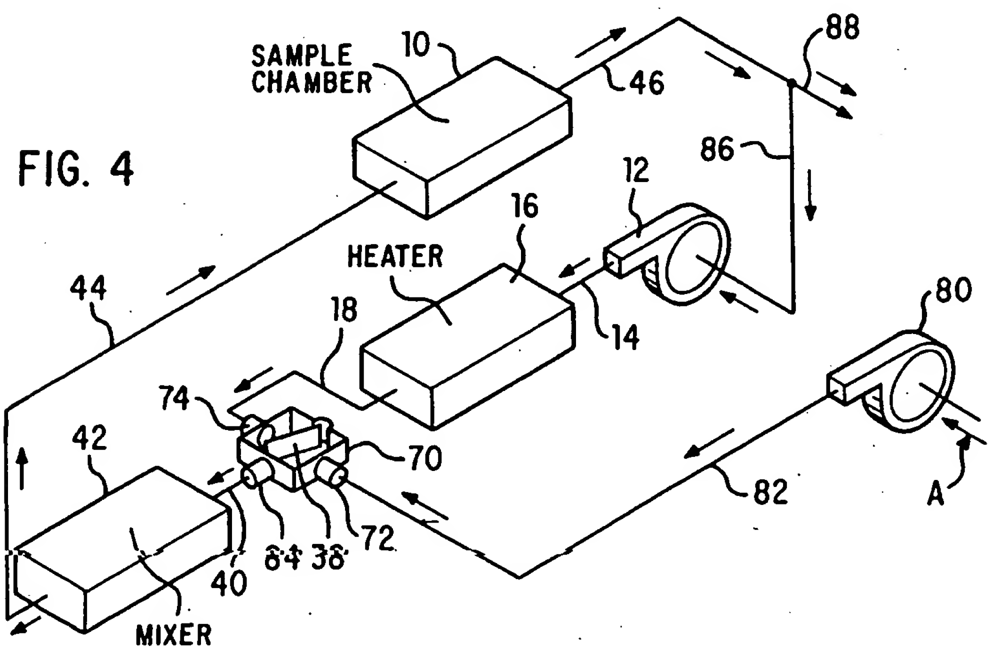

SAMPLE CHAMBER
HEATER
MIXER
10
12
14
16
18
40
42
44
46
70
72
74
80
82
86
88
A

FIG. 4

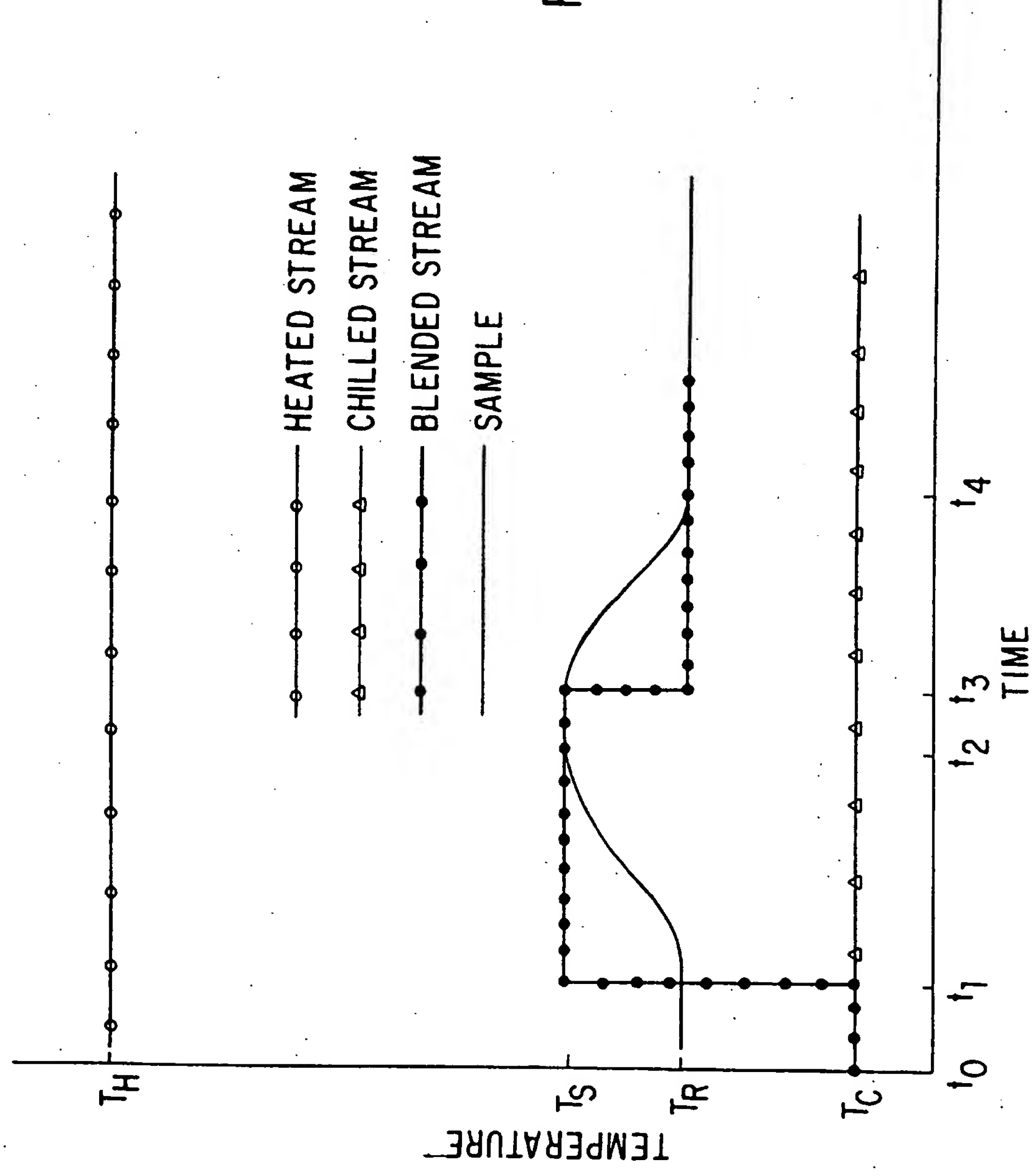
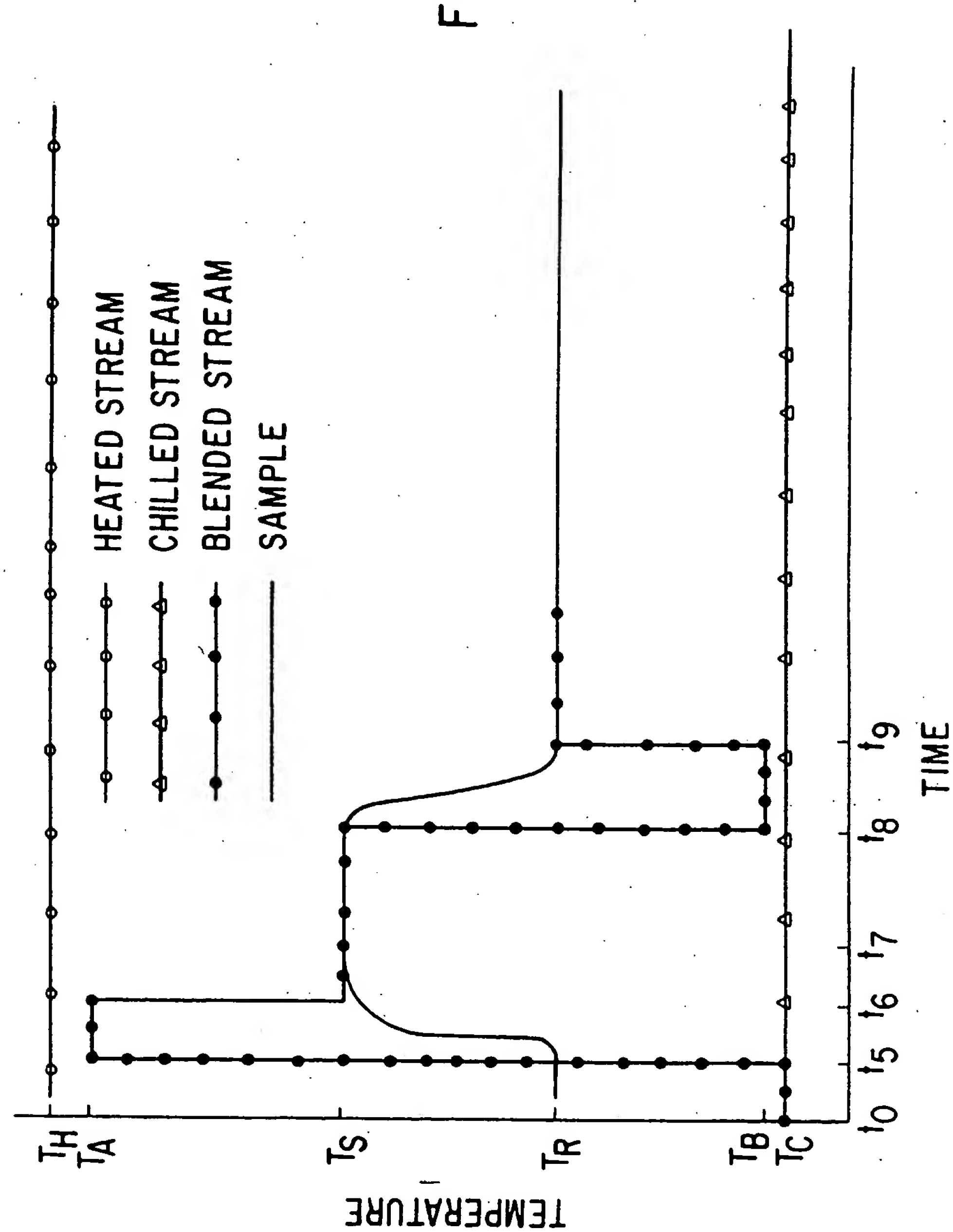

HEATED STREAM
CHILLED STREAM
BLENDED STREAM
SAMPLE
TEMPERATURE
T_H
T_S
T_R
T_C
TIME
t_0
t_1
t_2
t_3
t_4

FIG. 5

FIG. 6


HEATED STREAM
CHILLED STREAM
BLENDED STREAM
SAMPLE
TEMPERATURE
T_H
T_A
T_S
T_R
T_B
T_C
TIME
t_0
t_5
t_6
t_7
t_8
t_9